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RESEARCH **Open Access**

Intraspecific body size variability in a population of *Billaea rhynchophorae* (Blanchard) (Diptera: Tachinidae) parasitizing *Rhynchophorus palmarum* (Linnaeus) (Coleoptera: Curculionidae) in Brazil

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Abstract

Background The black palm weevil, *Rhynchophorus palmarum* (Linnaeus, 1758) (Coleoptera: Curculionidae) is considered the most important palm pest in the Americas. In the State of Bahia, Brazil, it is parasitized by tachinid flies (Diptera: Tachinidae). During 2019–2020, larvae and pupae of *R. palmarum* were collected from African oil palms (*Elaeis guineensis* Jacq.) in the municipalities of Jaguaripe and Taperoá, Bahia State, Brazil. Puparia and adult tachinids of two apparent morphospecies, with considerable differences in body size, were obtained from the *R. palmarum* immatures, which led to initially assume that we had collected two different tachinid species.

Results Morphological identification as well as *COI* gene sequencing showed that the two apparent morphospecies belong to a single taxonomic species, *Billaea rhynchophorae* (Blanchard, 1937) (Diptera: Tachinidae).

Conclusions Occurrence of intraspecific size variability in *B. rhynchophorae* was reported, as well as performed the first genetic sequencing for this species. The reason of the size variation could not be determined but some possible causes and implications are discussed.

Keywords Tachinids, *Rhynchophorus palmarum*, *Elaeis guineensis*, Superparasitism, Biological control

Background

The South American palm weevil, *Rhynchophorus palmarum* (Linnaeus, 1758) (Coleoptera: Curculionidae), infests several palm species (Arecaceae) of economic importance (Löhr et al. 2015), and it is responsible for killing thousands of ornamental and commercial palms like coconut palm (*Cocos nucifera* L.) and oil palm (*Elaeis guineensis* Jacq.) in North, Central and South America (Dalbon et al. 2019). The larvae feed inside the palm stem, destroying it, and the adults are the primary vector of *Bursaphelenchus cocophilus* Cobb (Baujard) (Ferreira et al. 2017), the nematode associated with the red ring

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disease, which is lethal to coconut and other palm species. Therefore, for a *R. palmarum* management program, it is necessary to adopt multiple complementary tactics, including cultural measures, biological control and use of semiochemicals (Dalbon et al. 2021). In this way, the development of control tactics for this pest is constant, which includes the search for natural enemies. Tachinids (Diptera: Tachinidae) have been found to parasitize *R. palmarum* in Brazil. In the state of Bahia, Northeastern region of Brazil, Moura et al. (1993) found *Paratheresia menezesi*, junior synonym of *Billaea menezesi* (Guimarães, 1977) on *R. palmarum* in *E. guineensis* plantations and Moura et al. (2006) found *Billaea rhynchophorae* (Blanchard, 1937) on the same pest in *E. guineensis* and *Attalea funifera* Mart. plantations (Arecaceae).

In recent surveys at the same region, pupae of *R. palmarum* were collected and tachinid puparia of two clearly distinct sizes were obtained. Host pupae contained only small or only big tachinid puparia, never a mixture. This led to initially assume that the same two species found by Moura et al. (1993; 2006) (*B. menezesi* and *B. rhynchophorae*, respectively) were collected. In order to confirm this hypothesis, morphological identifications and molecular sequencing of the two apparent morphospecies collected were performed.

Methods

From 2019 to 2020, pupae of *R. palmarum* in *E. guineensis* plantations, in the municipalities of Jaguaripe (13° 00′ 49.5″ S; 38° 51′ 08.3″ W; 19 m above sea level) and Taperoá (13° 31′ 30.9″ S; 39° 07′ 09.9″ W; 65 m above sea level), Bahia, Brazil, were collected. Both locations are approximately 63 km apart from each other. The climate in the region is tropical without dry season (Af in Koppe's classification). The annual rainfall is 1900–2200 mm and the annual mean temperature is 24–26 °C (Alvares et al. 2013). For both puparia sizes, the length, width, weight and number of tachinid puparia per host pupa were found and submitted to analysis of variance using the software SASM-Agri (Canteri et al. 2001). Puparia were kept in BOD chambers (25.0 ± 1 °C; 70 ± 10% RH) until adult emergence. For identification, some adults emerged were dry-mounted on pins and observed under a stereoscope, and male genitalia was also dissected and examined. Adult flies were identified based on Blanchard (1937) and Guimarães (1977). Morphological terminology followed Cumming and Wood (2017). Vouchers were deposited in the Zoological Reference Collection of the Universidade Federal de Mato Grosso do Sul (ZUFMS) in Campo Grande, Mato Grosso do Sul, Brazil.

To confirm insect identification, adult specimens were also conserved in alcohol 100% for genetic sequencing of the mitochondrial DNA (mtDNA). The total DNA from the entire body of insects of both sizes was extracted by Dneasy® Plant Mini Kit (Qiagen). The partial sequences of the mitochondrial cytochrome c oxidase subunit I gene (*COI*) were amplified by polymerase chain reaction (PCR) using the primers 2183 (CAACATTTATTTTGATTTTTTGG) and UEA8 (AAAAATGTTGAGGGAAAAATGTTA) according to Ceotto et al. (2008). After amplification, PCR products were electrophoresed in 2% agarose gel, stained with ethidium bromide, and visualized under UV light to confirm its size. The samples were then purified with GFX™ PCR DNA and Gel Purification kit (GE Healthcare®) and sequenced by ACT Gene Análises Moleculares Ltda (Brazil). The DNA from *Musca domestica* L., 1758 (Diptera: Muscidae) was also extracted and sequenced as described above, serving as an outlier. The *COI* sequence obtained for the tachinids was blasted against the GenBank database and checked in BOLD Identification System (Ratnasingham and Hebert 2007).

Results

All size parameters were significantly different between big and small puparia, and the number of tachinid puparia per host pupa was significantly higher when they were small than when they were big (Table 1). Adults that emerged from such puparia also exhibited distinct body sizes (Fig. 1). Small puparia resulted in small adult males and females (body length of approximately 8 mm), and big puparia resulted in big adult males and females (body length of approximately 12 mm).

Table 1 Mean (± SD) size parameters in puparia of *Billaea rhynchophorae* obtained from *Rhynchophorus palmarum* in *Elaeis guineensis*, in Jaguaripe and Taperoá, BA, Brazil (2019–2020)

Puparia size	Length (mm)*	Width (mm)*	Weight (g)*	Puparia per host pupa*
Big	11.15 ± 0.63[a]	5.14 ± 0.45[a]	0.08 ± 0.03[a]	11.80 ± 7.50[b]
Small	9.48 ± 0.70[c]	4.06 ± 0.38[c]	0.04 ± 0.02[c]	29.43 ± 19.33[d]

*All parameters were significantly different according to ANOVA

[a] $n = 181$

[b] $n = 94$

[c] $n = 85$

[d] $n = 49$

Fig. 1 Size variability in *Billaea rhynchophorae* adults emerged from puparia of different sizes obtained from *Rhynchophorus palmarum*. Scale bar = 5 mm

Both small and big flies were morphologically identified as *B. rhynchophorae*. This species can be recognized by the following combination of features: eye bare; head gray pruinose; vertex more than 0.23 head width in dorsal view; a low ridge not visible in profile on the midline of the face; pedicel with one long setae more than a half as long as arista; arista plumose; palpus not strongly clavate at tip; parafacial bare; thorax dark brown in ground color with gray pruninosity; mesonotum with three broad black vittae; prosternum bare; proepisternum haired; three katepisternal setae; katepimeron with a few hairs; abdomen orange brown to brown in ground color with gray pruinosity, pale gray at some angles, with irregular triangular areas without pruinosity mainly in tergites 3 and 4; syntergite 1 + 2 excavated medially to posterior margin; tergite 7 + 8 in profile large, wider than epandrium; surstylus in profile broad, elongated, and ogival; cercus slightly curved and narrowing from the middle toward the apex (Blanchard 1937; Guimarães 1977) (Fig. 2).

The *COI* sequences obtained for the tachinids of both sizes were identical, confirming that they belong to the same species, in this case, *B. rhynchophorae*, as morphologically identified. The sequence obtained for house fly was distinct from the former, demonstrating the suitability of the technique in distinguishing different fly species. The maximum similarity of *B. rhynchophorae* with species already deposited in GenBank was 83%. The sequence was then deposited in GenBank (OM151283), the first sequence for *B. rhynchophorae* in this database.

Discussion

According to Guimarães (1977), the identification of the species of the genus *Billaea* (as *Paratheresia* Townsend) is one of the most difficult problems in the taxonomy of Tachinidae due to the high intraspecific variations existing in samples from a single locality or even from the same hosts. The presence of intraspecific variation makes it difficult to find good characters for species identification (Emden 1949; Sabrosky *in* Emden *loc. cit.*; Guimarães 1977). Guimarães (1977) stated that *B. rhynchophorae* is very similar to *B. claripalpis* (Wulp 1896), from which it can differ mainly in having a robust size, broader front at vertex, and palpi not strongly clavate at tip. *B. claripalpis* exhibits great variation in its chaetotaxy, body size, coloration, and male terminalia (Emden 1949; Sabrosky *in* Emden *loc. cit.*; Guimarães 1977) and seems to be a species complex. Although the male terminalia is not the only reliable criterion for diagnosing the species in this group, it should always be considered when there are doubts in the identification procedure (Guimarães 1977). The male terminalia of the specimens studied matched the well-done illustration accompanying the original description of *B. rhynchophorae*, dispelling any doubts about the identification of the material. The terminalia of *B. rhynchophorae* differs from that of *B. menezesi* by the ogival surstylus and from that of *B. claripalpis* by the more elongated surstylus and the cercus narrowing from the middle to the apex.

Blanchard (1937), when describing *Parabillaea rhynchophorae* (= *B. rhynchophorae*), did not mention the species body size. However, Guimarães (1977), reviewing the genus, mentioned a total body length of 11–13 mm for *B. rhynchophorae*. Therefore, our big flies correspond to the already known size of the species, and the presence of the small form in the population can be considered as dwarfism.

Tachinidae is one of the most ecologically important families in the order Diptera. As parasitoids, they are important natural enemies, particularly in terrestrial ecological communities. Most tachinid species parasitize holometabolous insect larvae, playing an important ecological role by regulating populations of herbivorous insects in both natural and managed ecosystems (Stireman et al. 2006; 2019).

Parasitoids can present a high intraspecific size variability, which has been shown to depend on several host factors such as species, sex, stage, weight, and volume (Salt 1941). For tachinids, intraspecific size variability in *Pseudogonia rufifrons* (Wiedemann, 1830) (Diptera: Tachinidae) was shown to be related to host body size (Baronio et al. 2002). However, the work refers to two host species with different body sizes. Intraspecific size variability in the same host species has been shown to be related to the age of the host at the moment of parasitization (Mellini and Baronio 1971; 1972; Baronio et al. 1981). For *Cleonice callida* (Meigen, 1824) (= *Steiniella callida*) (Diptera: Tachinidae) parasitizing *Chrysomela populi* L., 1758 (Coleoptera: Chrysomelidae), the weight and volume of the puparia do not vary appreciably when parasitization occurs in the first or second larval instars of the host, but there is considerable dwarfism if parasitization occurs during the second

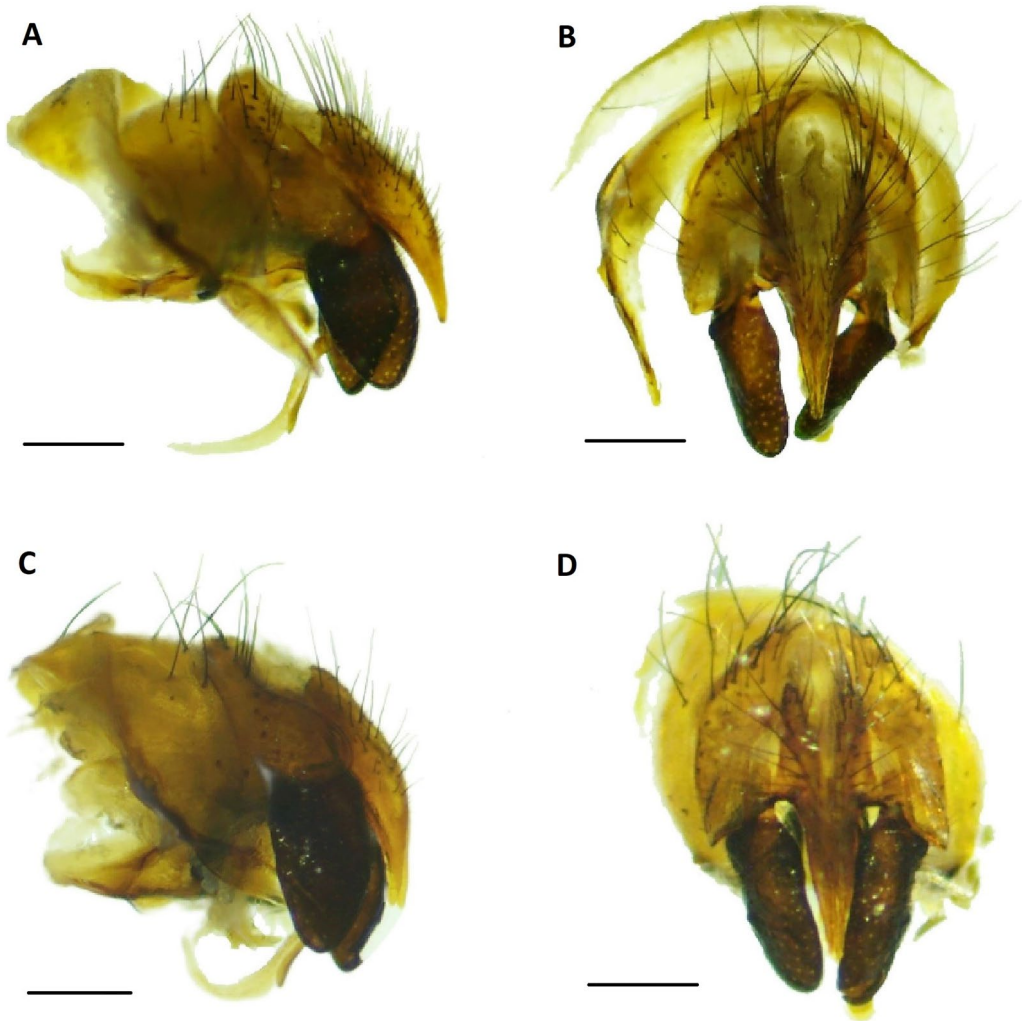

Fig. 2 *Billaea rhynchophorae* male terminalia: **A** big specimen, lateral view; **B** big specimen, posterior view; **C** small specimen, lateral view; **D** small specimen, posterior view. Scale bars = 0.25 mm

half of the third larval instar of the host (Mellini and Baronio 1971). Parasitization in the early prepupal stage of the host also resulted in dwarfing of the parasitoids, with size decreasing progressively as the age of the host at parasitization increases (Mellini and Baronio 1972).

According to Welch (2006), reduced size is the easiest observable type of fitness reduction in the tachinid *Ormia depleta* (Wiedemann, 1830), and can be caused by superparasitism, which is multiple parasitization of a host by the same parasitoid species. Due to competition, insufficient resources are available to allow the flies reared under such conditions to develop, mate, locate hosts, or reproduce properly. There is no evidence that tachinids are able to discriminate an already parasitized host (Caron et al. 2010), which favors superparasitism in the family. Besides the nutritional status represented by resources allocation during development, reduced body size can also influence insect behavior and fitness in the field. Small individuals have a higher body surface-to-volume ratio, making them more susceptible to thermal changes with shifts in ambient temperature and to water loss due to transpiration, so that thermoregulatory capacity of insects is often a function of body mass (Brito et al. 2020).

On the other hand, insect dwarfism can be understood as a positive trait in some cases. Reduction in body size can reflect the ability of some parasitoid species to adapt its development to a smaller factitious host in a perspective of mass production for releasing them in biological control programs (Baronio et al. 2002).

Conclusion

It was demonstrated that there is an intraspecific body size variability in *B. rhynchophorae*. The cause of the size variability observed in the population of the present work

could not be determined, and further studies are necessary to elucidate this. The effect of such dwarfism on the fitness of individuals in nature is still under investigation. Genetic sequencing of *B. rhynchophorae* was also performed for the first time, contributing with the knowledge about this species.

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Author contributions
ECG, ASNJ, BLL, JGV, JPMA, TTS and DJS collected the insects. RT identified species. LECD performed molecular analyses. ECG, LECD and RT wrote the first draft of the manuscript. All authors commented on previous versions, read and approved the final version of the manuscript.

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Availability of data and materials
The voucher material of adult *Billaea rhynchophorae* studied is deposited at the Zoological Reference Collection of the Universidade Federal de Mato Grosso do Sul (ZUFMS), in Campo Grande, Mato Grosso do Sul, Brazil. The genetic sequence obtained from this study was deposited in GenBank (www.ncbi.nlm.nih.gov/genbank) under the accession number OM151283.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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